Attorney Docket No.: ALX-141 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Yi Wang, Louis Matis and Scott Rollins

Serial No.: 08/236,208

Filed : May 2, 1994

For : METHOD FOR THE TREATMENT OF GLOMERULONEPHRITIS

Examiner : Phillip Gambel, Ph.D.

Group : 1806

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION OF LOUIS A. MATIS PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Louis A. Matis, hereby declare that:

1. I am an inventor of the invention claimed in the above-identified application and have read the office action in the application dated July 25, 1995.

2. I received a B.A. in Liberal Arts from Amherst College, Amherst, MA in 1971. I received an M.D. in 1975 from the University of Pennsylvania Medical School, Philadelphia, PA. For the past 16 years, I have been involved in immunological research, first at the National Institute of Allergy and Infectious Disease, National Institutes of Health, then at the Center for Biologics Evaluation and Research of the Food and Drug Administration, and subsequently at the National Cancer Institute, National Institutes of Health. I am now Vice President, Research - Immunobiology at

Alexion Pharmaceuticals, Inc., New Haven, CT, the licensee of the above-identified application, where I have been employed since January, 1993. I am also currently visiting lecturer, Section of Immunobiology, Yale University, New Haven, CT.

3. I understand that the Examiner has asserted that it would be obvious to treat glomerulonephritis (GN) in accordance with the methods of the above-referenced application in view of various publications suggesting that terminal complement components play an important role in the development of the disease. I believe that this assertion is in error, as the reasoning leading to this assertion does not take into account relevant and important facts that are well known in this field of research. These facts, and their impact on the expectations of researchers in this field, are discussed below in paragraphs 4-6.

4. GN is typically associated with the deposition of immune complexes in the kidney glomeruli. In most cases these complexes are pre-formed outside of the kidney, are carried throughout the body by in the blood, and are preferentially deposited in the kidney as a result of the filtration function of this organ. The administration of an antibody reactive with a plasma protein (such as complement component C5) would be likely to result in the formation of circulating immune complexes (which would be likely to be deposited in the kidneys). The administration of antibodies specific to such plasma proteins (e.g., in accordance with the methods of the above-referenced application) would thus be generally expected to promote or exacerbate GN. Therefore, workers in this field of research would not find it obvious to

treat GN by the administration of any monoclonal antibody, because, regardless of any other effects, such treatment would be expected to promote immune complex deposition in kidney glomeruli, which would be expected to accelerate the disease process.

5. If terminal complement activation products were the only proinflammatory factors known to effect the glomerular inflammation of immune complex mediated GN, it could, perhaps, be argued that the deposition of immune complexes expected following anti C5 antibody administration might not have been predicted to promote disease, because complement blockade alone might have been expected to prevent this result. This, however, is most certainly not the case. The scientific literature in the area of immune complex mediated GN is replete with reports of a wide variety of proinflammatory factors that have been associated with pathogenesis following immune complex deposition. Such factors as interferons, interleukins, chemokines, tumor necrosis factors, early complement components (e.g., C3), colony stimulating factors, and other growth factors, have all been implicated as playing important roles in the pathogenesis of immune complex mediated GN. Even in the absence of terminal complement function, these other proinflammatory factors would have been expected to promote GN upon the deposition of immune complexes in the glomeruli. This expectation would have been supported by the knowledge that mice bred to carry a genetic lesion resulting in a complete absence of complement component C5 have been reported to develop GN similar to that seen in mice with normal C5 levels (see the discussion of NZB nephritis below, particularly paragraph 10).

6. Thus, it is arguable whether there would have been any expectation of success in treating GN by blocking any particular one of the many proinflammatory factors associated with immune complex mediated GN, and, more to the point, there would certainly not have been a reasonable expectation of success in treating GN by the blockade of terminal complement activation by any means (since animals without terminal complement function still get the disease), much less by a method that would be expected to promote further immune complex deposition in the kidney. In view of the foregoing, no one knowledgeable in the field of GN research would have found it obvious (without knowledge of the results reported in the examples of the above-referenced application) to suppose that blockade of complement component C5, a single proinflammatory factor, under conditions that would be expected not to inhibit immune complex deposition (an expectation confirmed by the data of Example 3 of the above-referenced application) -- conditions that, in fact, would be expected to exacerbate immune complex deposition -- would have a significantly beneficial effect on kidney disease.

7. I understand that the Examiner has raised a question regarding the *in vivo* efficacy of complement inhibition using anti-C5 monoclonal antibodies. I believe that the results of the experiments of the examples of the above-referenced application, together with the pharmacokinetic data discussed in the Declaration of Scott A. Rollins, submitted herewith, and the *in vivo* "NZB nephritis" data described below, show that anti-C5 monoclonal antibodies are highly effective *in vivo*.

8. The *in vivo* data presented in the examples of the above-referenced application were obtained using an accepted animal model of human disease in which GN is induced in mice by the injection of foreign proteins that stimulate immune complex deposition in kidney glomeruli ("HAF nephritis"). Additional data were obtained using another accepted animal model of human disease, in this case a murine model in which there is a genetic predisposition to the development of a disease state analogous to human systemic lupus erythematosis (SLE). In mice with this genetic predisposition, the course of the inherited disease culminates (as untreated human SLE often does) in severe, and ultimately fatal, immune complex mediated GN ("NZB nephritis").

9. New Zealand black x New Zealand white (NZB/W) hybrid F_1 mice spontaneously develop an autoimmune syndrome with striking similarities to human SLE. Female NZB/W F_1 mice produce high titers of IgG anti-nuclear antibodies, including antibodies to double-stranded DNA (dsDNA), and invariably succumb to severe immune complex-mediated GN that results in death from renal failure in virtually all animals by 12 months of age. These mice thus serve as an accepted model for human SLE, and, in particular, NZB nephritis serves as an accepted model for human immune complex-mediated lupus nephritis (GN).

10. Although the development of lupus nephritis is accompanied by abundant local and systemic complement activation, the role of terminal complement components in disease progression has not been established. In NZB/W F_1 mice, as in human SLE, the production of autoantibodies and consequent tissue deposition of

immune complexes result in local and systemic complement activation sufficient in magnitude to cause a marked reduction in serum complement-dependent hemolytic activity. This observation, taken alone, would seem to implicate the complement system as playing some role in this disease. However, studies in NZB- and NZW- derived mouse strains lacking the C5 component of complement have shown that such mice will still develop immune complex-mediated GN, even though they cannot make functional terminal complement effectors. The development of this disease in animals with a lesion in the terminal complement pathway has thus cast doubt on the role of terminal complement activation in the progression of immune complex-mediated GN, and, particularly on whether terminal complement is a critical factor in pathogenesis.

11. Studies undertaken under my supervision at Alexion Pharmaceuticals have directly examined the involvement of terminal complement in the pathogenesis of the GN in C5 sufficient NZB/W F_1 mice, using the BB5.1 mAb specific for murine C5 used in several of the examples of the above-referenced application. The data obtained in these studies show that continuous treatment with an anti-murine C5 mAb results in marked amelioration of the course of renal disease and in dramatic prolongation of survival. The following paragraphs detail the Materials and Methods (M&M), and Results of these studies. The figures referred to in the Results, and the legends therefor, are appended hereto as Exhibit A.

12. *M&M - Animals.* 8-12 week old female NZB/W F_1 mice were purchased from the Jackson Laboratory, Bar Harbor, ME, and were maintained under pathogen-free conditions.

13. *M&M - Antibodies and treatment.* Antibodies used were anti-mouse C5 mAb BB5.1 and murine anti-human C8 mAb 135.8, which does not inhibit murine complement (as a control). Both mAbs were from ascites from hybridomas grown in athymic mice, purified by protein A affinity chromatography followed by elution with IMMUNO PURE IgG elution buffer (Pierce, Rockford, IL) and dialysis against Tris-buffered saline. Concentrations of purified antibodies were determined at O.D. 280 with a Beckman DU-640 spectrophotometer. At 18 weeks of age, mice were begun on biweekly treatments with 1 mg of either anti-C5 or control mAb administered intraperitoneally. Beginning at 26 weeks the frequency of treatments was increased to three times per week and from 32 weeks onward daily treatments were initiated. Mice were bled every two weeks for determination of serum hemolytic activity. Hemolytic assays were performed essentially as described in the above-referenced application. The 100% value for complement-dependent serum hemolytic activity was determined using normal mouse serum (Sigma, St. Louis, MO). The serum hemolytic activity of the anti-C5 mAb treated mice was continuously maintained at a level less than 10% of that of normal mouse serum.

14. *M&M - Assays.* Urine protein levels were determined three times per week by colorimetric analysis using dipsticks (CHEMSTRIP 2GP, Boehringer Mannheim Diagnostics, Indianapolis, IN). and quantitated according to the following parameters: trace; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 500 mg/dl. Baseline levels of anti-dsDNA antibodies, measured using a standard ELISA assay, were determined using sera from 5 week old NZB/W F_1 mice

prior to the onset of signs of autoimmune disease. The serum titers of antibodies to dsDNA in NZB/W F_1 mice measured at subsequent time points (18 and 32 weeks) were then recorded as the fold increase in the number of O.D. units of specific antibody relative to the baseline measurements at 5 weeks.

15. *M&M - Renal histopathology.* The kidneys from control mAb-treated, anti-C5 mAb treated, and young untreated mice were fixed in 10% buffered formalin. The tissue was then processed and embedded in paraffin. Tissue sections (5 mm) were stained with periodic acid Schiff (PAS) or hematoxylin/eosin (H&E) using standard methodology.

16. *M&M - Mesangial Matrix analysis.* Quantitative analysis of mesangial matrix deposition, a representative feature of the renal histopathology in the NZB/W F_1 model of lupus GN, was performed on glomeruli from 15 control-mAb-treated mice, 17 anti-C5 mAb-treated mice, and 6 untreated 18 week old NZB/W F_1 mice (to determine the average matrix volume at the time treatment was begun). Kidney sections were processed from euthanized mice, and the volume of the mesangial matrix was measured in ten randomly selected H & E-stained glomeruli per animal. Images were captured using a JVC TK-1070U video system through a 40x objective lens and were quantitated with IMAGE-PRO PLUS software (Media Cybernetics, Silver Spring, MD). Specifically, the perimeter of the glomerular mesangial matrix from each H & E-stained glomerulus was traced and the area determined by performing pixel counts. Mesangial cell areas were automatically excluded from the measurements using the

color differentiation parameters of the count/size command of the software.

17. *RESULTS - Inhibition of complement in NZB/W F_1 mice.* To examine the role of activated terminal complement components in the progression of autoimmune disease in NZB/W F_1 female mice, 4 month old animals were begun on biweekly treatments with either anti-C5 or an isotype matched control mAb. The BB5.1 anti-C5 mAb blocks the generation of both C5a and C5b-9. *In vivo* inhibition of complement by anti-C5 mAb was ascertained by serial measurement of complement-dependent serum hemolytic activity. The mAb treatments, continued until the mice were 40 weeks of age, were titered thereafter to maintain serum hemolytic activity at a level less than 10% of that of normal control mouse serum (Figure 1A). At the time of initiation of therapy, all mice had low but measurable levels of circulating anti-dsDNA antibodies (Figure 1B). Anti-C5 mAb administration was able to sustain complement inhibition *in vivo* for the entire 6 month period of treatment, as measured by reduced serum hemolytic activity (Figure 1A). In contrast to anti-C5 mAb treated animals, the serum hemolytic activity of the control mAb-treated mice was normal at the outset of the study and then gradually declined to less than 10% of normal levels by 40 weeks of age, presumably secondary to systemic consumption of complement following widespread tissue deposition of immune complexes (Figure 1A). The decline in hemolytic activity of the control sera correlated with elevated titers of anti-dsDNA antibodies measured over the same period of time

(Figure 1B). Both anti-C5 and control mAb-treated mice produced comparable amounts of anti-dsDNA antibodies (Figure 1B).

18. *RESULTS - Amelioration of immune complex GN and prolongation of survival by anti-C5 therapy.* The influence of mAb-mediated C5 inhibition on the course of NZB nephritis was examined both clinically and histopathologically. A marked delay in the onset of severe proteinuria, defined as equal to or greater than 500 mg/dl (≥3+), was achieved in anti-C5 mAb treated mice relative to controls (Figure 2A). Whereas all of the control mice had developed proteinuria accompanied by marked total body edema by 32 weeks, no anti-C5 mAb treated animals developed proteinuria until 33 weeks of age, and a significant percentage of these mice maintained normal renal function without evidence of proteinuria throughout the treatment period (Figure 2A). Coincident with ameliorating the clinical signs of severe immune complex nephritis, C5 inhibition was associated with a dramatic prolongation of survival. Over 80% of anti-C5 mAb treated mice were still alive at 40 weeks, in contrast to <5% of animals treated with control mAb (Figure 2B). Very similar results were obtained in a separate experiment performed on an independent group of NZB/W F_1 mice.

19. *RESULTS -Histopathology.* Histopathologic examination was performed on renal tissue from anti-C5 treated, control mAb treated, and young untreated animals (Figure 3). Figure 3A illustrates a representative glomerulus from a normal appearing PAS stained kidney of an 18 week old NZB/W F_1 mouse prior to the onset of signs of renal disease. In this group of animals the

tubular and glomerular basement membranes appear as thin delicate continuous ribbons, and the tubule lumina are free of casts. Figure 3B is representative of glomeruli from control mAb treated mice with ≥3+ urine protein, characterized by dramatic changes in the mesangial matrix. There is considerable expansion of the mesangial matrix with amorphous PAS staining material (Figure 3B). In addition, in these kidneys many tubule lumina are filled with PAS positive casts. In contrast, examination of renal tissue from age-matched anti-C5 mAb treated mice without detectable proteinuria revealed normal appearing tubular and glomerular basement membranes, with the renal tubules free of casts and only minimal mesangial expansion (Figure 3C). Interestingly, analysis of renal tissue from the few anti-C5 mAb treated mice with ≥ 3+ proteinuria also demonstrated essentially normal tubular and glomerular architecture, with the exception of only modestly increased PAS positive mesangial matrix deposition (Figure 3D). Examination of H & E stained sections of kidneys from control mAb treated animals confirmed the mesangial matrix expansion described above. In contrast, H & E stained glomeruli of the anti-C5 mAb treated mice with ≤1+ proteinuria exhibited only minimal increases in mesangial matrix deposition. Again, H & E stained glomeruli of the anti-C5 mAb treated mice with ≥3+ proteinuria manifested only modest increases in mesangial matrix deposition. A systematic quantitation of the mesangial matrix deposition in the glomeruli of control mAb versus anti-C5 mAb treated mice was performed by examining multiple glomeruli from each group of animals. As demonstrated in Figure 4, the amount of mesangial matrix

deposition in C5-inhibited mice with normal renal function (≤1+ proteinuria) was markedly reduced compared to control mAb-treated mice ($p.<.001$), and was only modestly increased relative to the volume of mesangial matrix in normal glomeruli from young NZB/W F_1 mice. Stratification of anti-C5 mAb treated mice into groups having low (≤1+) versus high (≥3+) levels of urine protein showed less mesangial matrix expansion in the treated animals without proteinuria ($p.<.01$) compared to those with significant levels of urine protein. Furthermore, consistent with the examination of the histologic sections (Figure 3), this analysis also revealed that even those anti-C5 mAb treated animals with ≥3+ proteinuria had considerably less expansion of the mesangial matrix than control mAb treated animals excreting equivalent amounts of urine protein ($p<.001$) (Figure 4). Thus, the data in Figures 3 and 4 demonstrate that complement inhibition at C5 significantly reduces the severity of the renal histopathologic lesion, even in the presence of proteinuria.

20. The data obtained in the studies involving both the HAF nephritis and the NZB nephritis animal models consistently indicate that the methods of the invention of the above-referenced application are effective in treating GN *in vivo*. Taken together with the monkey data set forth in the Declaration of Scott Rollins (submitted herewith), the results of these animal model studies demonstrate that only standard procedures are needed for persons of ordinary skill in the art to effectively use anti-C5 antibodies in the treatment of GN in accordance with the disclosure of the above-referenced application.

21. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Louis Matis

Louis A. Matis, M.D.

Signed at New Haven, Connecticut
this 12th day of APRIL, 1996.

Exhibit A

FIGURE LEGENDS

Figure 1. A. In vivo inhibition of complement by anti-C5 mAb administration. Female NZB/W F_1 mice were treated with anti-C5 or isotype matched control mAb beginning at 18 weeks of age, as described in Materials and Methods. Sera from both treatment and control groups were tested for complement dependent hemolytic activity every 14 days until 40 weeks of age. B. Progressive age-related elevation of serum anti-dsDNA antibody titers in NZB/W F_1 mice. Antibody titers (O.D. units) were determined at 18 and 32 weeks, and recorded as fold increase relative to titers in 5 week old mice, as described in Materials and Methods. A. and B. (•-•), control mAb treated; (o-o), anti-C5 mAb treated.

Figure 2. A. Anti-C5 mAb therapy delays onset of proteinuria in NZB/W F_1 mice. Serial measurements of urine protein in both control and anti-C5 mAb treated animals were performed as outlined in Materials and Methods. The data are presented as percentage of animals with ≥3+ urine protein. Control mAb treated, (•-•); anti-C5 mAb treated, (o-o). B. Prolongation of survival following anti-C5 mAb treatment of NZB/W F_1 autoimmune disease. Shown are the % surviving animals in anti-C5 treated (n=13) and control mAb treated (n=20) groups of animals at different ages. Anti-C5, (—); control, (--).

Figure 3. Histopathologic examination of kidneys from anti-C5 mAb treated and control female NZB/W F_1 mice. Representative PAS stained kidney sections are shown from the following animals: A. Eighteen week old mice prior to the onset of autoimmune disease, illustrating normal glomerular architecture. B. Control mAb treated animals with ≥3+ proteinuria, showing extensive mesangial matrix deposition and diffuse glomerular basement membrane thickening. C. Anti-C5 mAb treated mice with ≤1+ proteinuria, illustrating normal glomerular basement membranes and a minimal increase in mesangial matrix deposition. D. Anti-C5 mAb treated mice with ≥3+ proteinuria, demonstrating normal appearing glomerular basement membranes and a modest increase in mesangial matrix deposition. Magnification: x200

Figure 4. Quantitation of mesangial matrix deposition in glomeruli from control mAb treated (n=15), anti-C5 mAb treated (≥3+ urine protein, n=10), anti-C5 mAb treated (≤1+ urine protein, n=7), and untreated 18 week old female NZB/W F_1 mice (n=6). Kidney sections were processed from euthanized animals and ten randomly selected glomeruli were analyzed per mouse. Matrix volumes were determined as described in the Materials and Methods and are represented as mean pixel counts. Vertical bars represent standard errors of the mean. Statistically significant differences between the groups were confirmed by the Student's two-tailed *t* test.

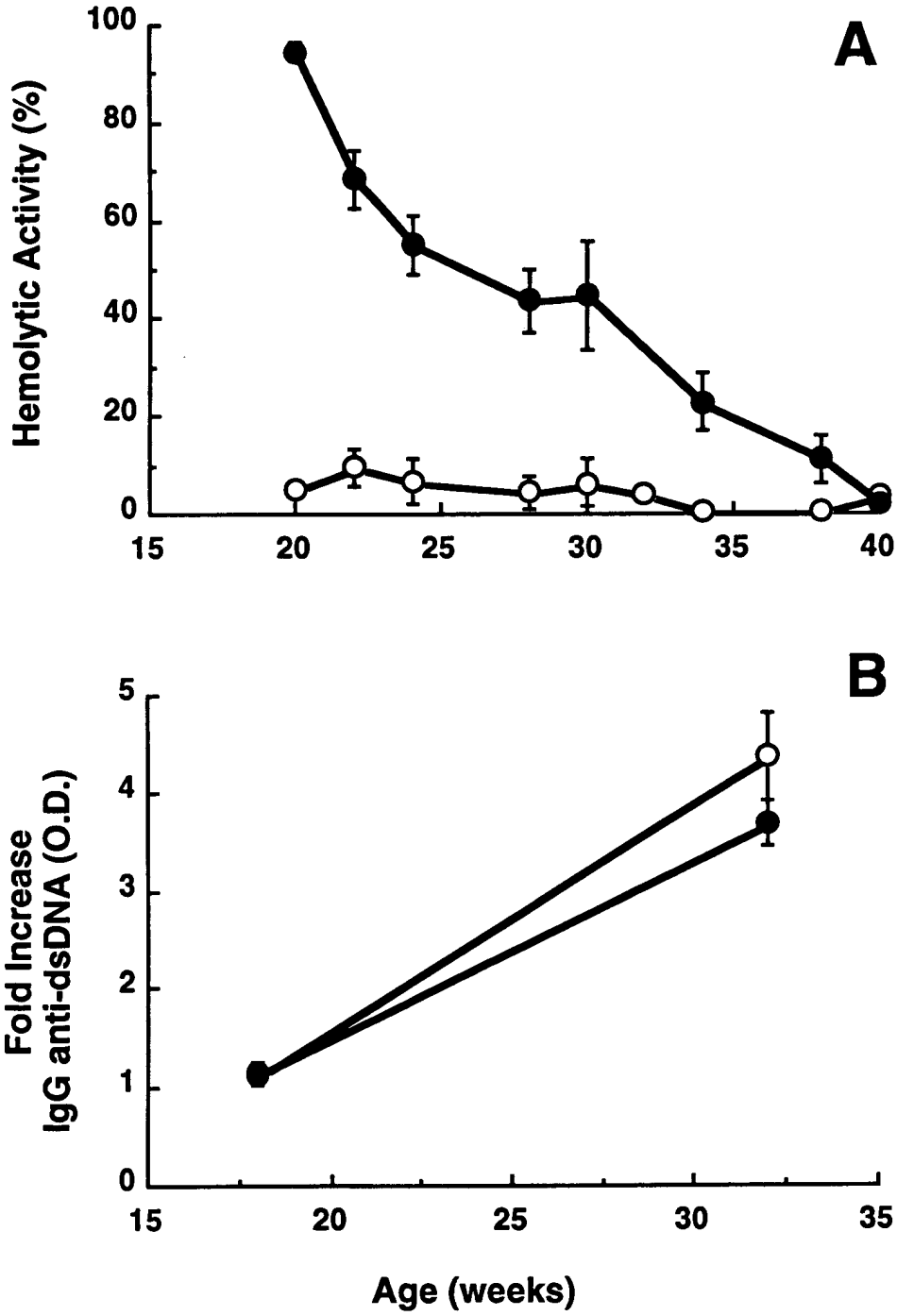
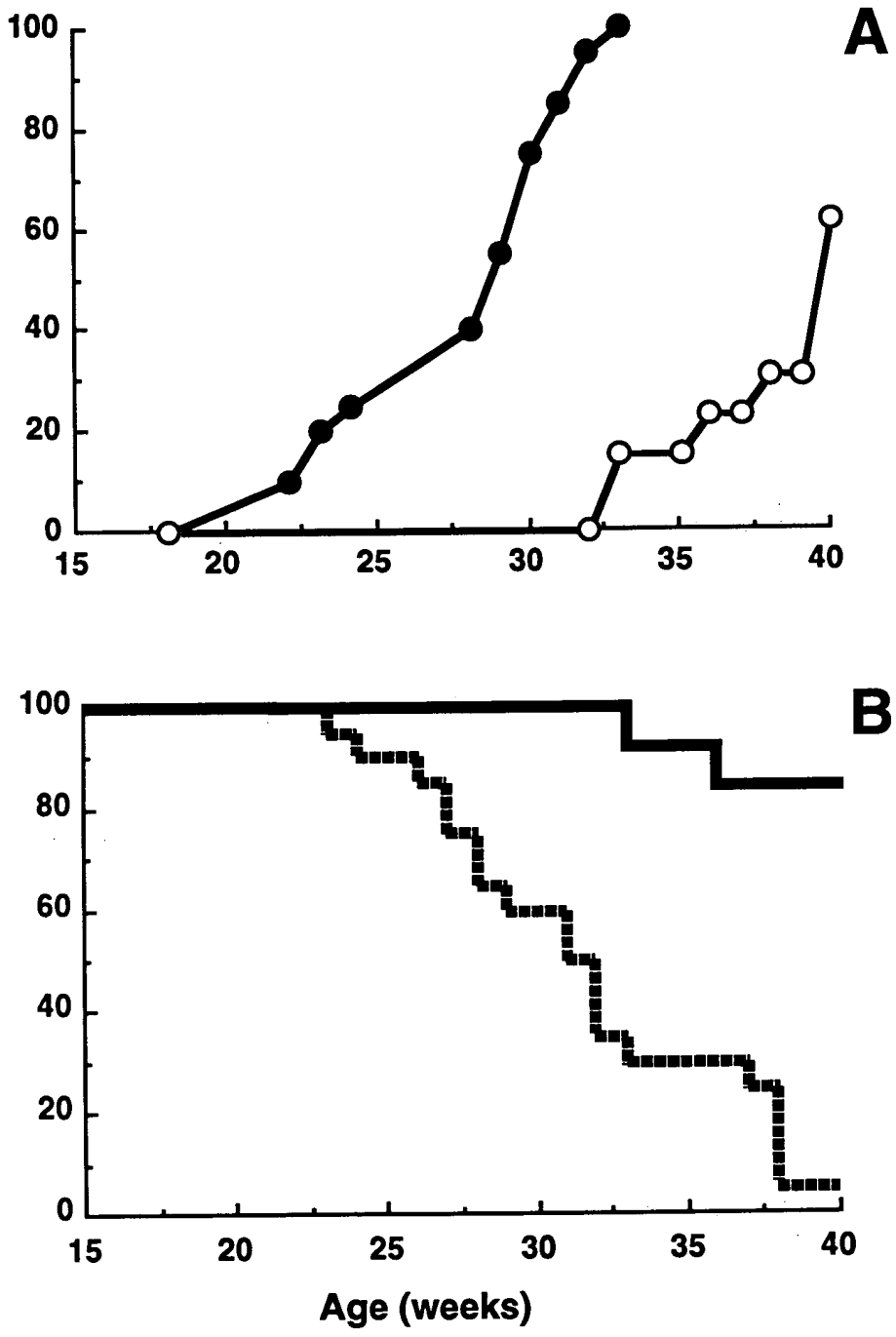

A
Hemolytic Activity (%)
100
80
60
40
20
0
15
20
25
30
35
40
B
Fold Increase
IgG anti-dsDNA (O.D.)
5
4
3
2
1
0
15
20
25
30
35
Age (weeks)

Figure 1


A
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Age (weeks)

Figure 2

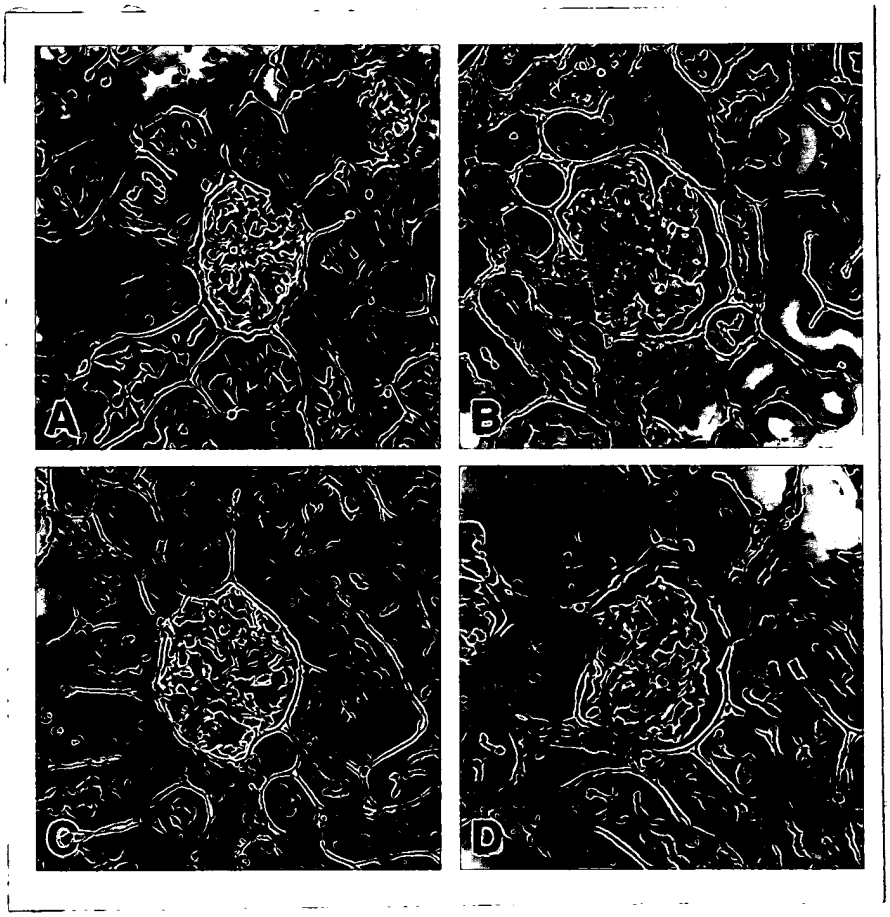

Figure 3

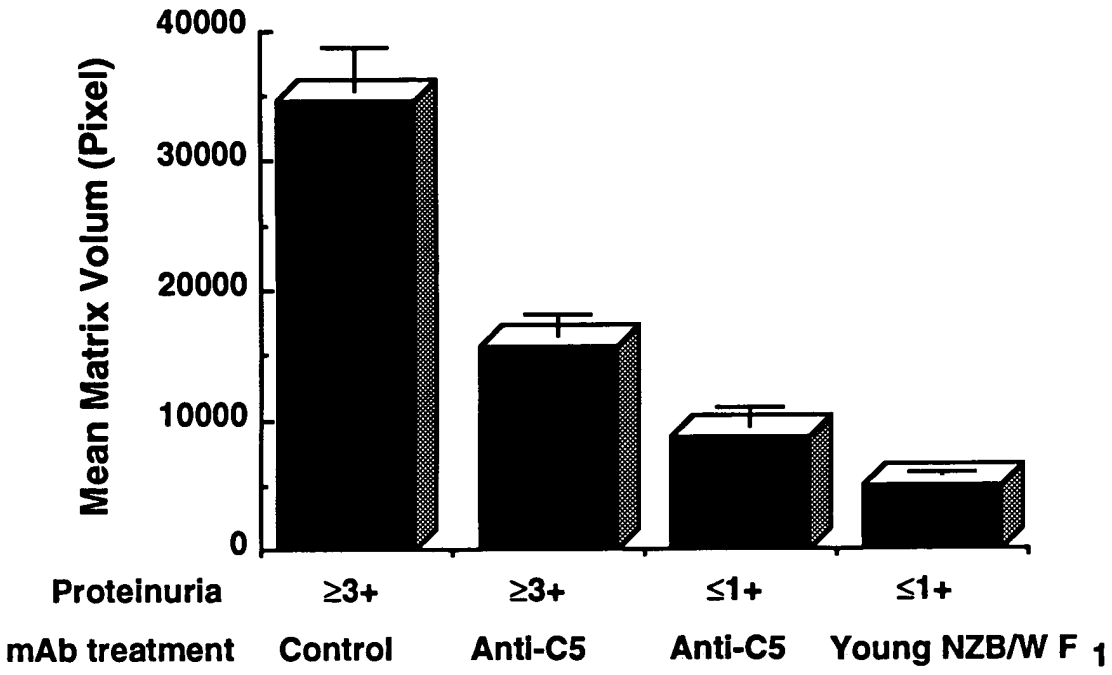
Mean Matrix Volum (Pixel)
40000
30000
20000
10000
0
Proteinuria
≥3+
≥3+
≤1+
≤1+
mAb treatment
Control
Anti-C5
Anti-C5
Young NZB/W F 1

Figure 4